Germicidal Activity Of Soaps

A study of the comparative germicidal value of rosin soaps and soaps of individual fatty acids

By L. S. Stuart and W. D. Pohle

THE germicidal activity of commercial soaps and the soaps of the individual fatty acids has been the subject of many investigations since Koch (9) first pointed out in 1881 their apparent antibacterial action. A complete critical review of the literature on the germicidal action of soap is beyond the scope of this investigation. Klarmann (8) in 1933 published a review of the work done up to that time in which he attempted to gather the widely scattered information found in the scientific, technical and trade journals, and subject it to a critical analysis. This was a monumental task and it provides some fairly concrete conceptions with regard to the germicidal activities of soaps. (Ed. Note. See also "Are Soaps Germicidal," *Soap and Sanitary Chemicals*, Jan., 1941, p. 23.)

There are no reports devoted primarily to rosin soaps or soaps containing rosin, although a few investigators have reported tests on sodium resinate and products bearing the name, at least, of sodium abietate. Others have reported results of tests made on brown bar (11) laundry soaps, which usually contain considerable sodium resinate, and the summation of these reports would indicate a possible germicidal superiority of sodium resinate and soaps containing rosin over fatty acid soaps. Using *Staphylococcus aureus* as a test organism, Walker (17, 18, 19) found sodium resinate to have greater germicidal activity than soaps of the common acids present in soap oils and fats such as caprylic, lauric, myristic, palmitic, stearic, oleic, and lineolic acids. With the fatty acid soaps, using selected strains of pneumococci and streptococci as test organisms, he found the intensity of the bactericidal action increased with increasing molecular weight of the acid up to lauric acid. Acids higher in the series than lauric produced soaps of less germicidal potency. *Eberthella typhi* was found to be more susceptible to the action of soaps of the saturated acids such as lauric and myristic, occurring in coconut oil, than to soaps of the unsaturated acids. His results with sodium resinate when *Eberthella typhi* was used showed this soap to have an activity only slightly less than that of coconut oil soap at a temperature of 20° C. and equivalent to that of coconut oil soap at 35° C.

The observation by Walker (18) that resin soap was much more effective against *Staphylococcus aureus* than fatty acid soaps, and almost equally as effective as coconut oil soap against *Eberthella typhi*, seems to have attracted no attention.

The germicidal activity of rosin soaps has been given primary consideration in this investigation. Effort has been directed toward the establishment of the relative bactericidal activity of rosin soaps and fatty acid soaps, the determination of the relative bactericidal activities of soaps made from rosin acids found in and derived from rosin and the examination of the factors that may influence the bactericidal activity of rosin soaps either alone or blended with the fatty acid soaps. This study was undertaken as a part of the investigational program of the Naval Stores Research Division designed to determine the properties contributed to soaps by the soaps of rosin and rosin acids, rosin and modified rosin.

For brevity the term "rosin soap" will be used to designate any soap made from rosin or rosin acids. All soaps made from an individual fatty acid or mixed fatty acid will be designated as "fatty acid soaps." "Soaps containing rosin" will refer to all soaps containing both fatty acid and rosin soaps.

In the past some have considered rosin to be 90 per cent abietic acid. This does not appear to be so (13), and some confusion in the literature has resulted from calling sodium resinate or rosin soap sodium abietate. The term abietic acid will be applied only to the rosin acid formed by acid isomerization of rosin acids and purified by recrystallization of the quarter salt of abietic acid.

Rosins contain 5 to 10 per cent of neutral material (sometimes referred to as resenes) and unless special steps are taken the sodium resinate or rosin soap will contain this neutral material. Rosins may also vary in composition with respect to the various kinds of rosin acids and amounts of oxidized rosin acids present. Sodium resinate will vary, therefore, in composition, depending upon the rosin from which it is made. Since rosin is produced by steam distilling gum obtained from both

* Mr. Stuart is associate bacteriologist, Eastern Regional Research Laboratory, and Mr. Pohle is assistant chemist, Naval Stores Research Division, Bureau of Agricultural Chemistry and Engineering, U. S. Dept. of Agriculture.

the longleaf and slash pine, rosins made from these two gums will be considered separately. The amount of oxidized resin and rosin acids in rosin depends upon the conditions of collecting and handling the gum and the subsequent treatment the rosin receives. In studying the effect of the presence of oxidized rosin acids on the germicidal activity of a rosin soap, a rosin was oxidized far more than the commercial gum rosins in order that the influence of the soaps of oxidized rosin acids might more easily be detected.

Pyroabietic acid, a stable product derived from rosin, was selected for comparison with normal gum rosin. Pyroabietic acid contains dehydroabietic acid, dihydroabietic acid, and tetrahydroabietic acid (6).

To determine the effects of differences in the structure of the rosin acids on the germicidal activity of their corresponding soaps, rosin acids containing varying amounts of hydrogen in the molecule were studied.

The rosins, rosin acids, derived rosin acids, fatty acids, and natural vegetable oil acids selected for the preparation of test soap solutions are listed together with pertinent information relative to chemical composition, special methods of preparation, collection or source of each selected products as follows:

Rosin Products

Slash Rosin—Rosin from slash pine gum collected in raised clay cups, using aluminum aprons.

Longleaf Rosin—Rosin from longleaf pine gum collected in raised clay cups, using aluminum aprons.

Oxidized Rosin—Longleaf rosin powdered and exposed to air in thin layer for over six months.

Pyroabietic Acids — Prepared from abietic acid by heating to 250° C. and using Pd-C for a catalyst (5).

Dehydroabietic Acid—Separated from pyroabietic, m.p. 170° C.; $[\alpha]_D^{20}$ +61°. (4)

Abietic Acid—Prepared by isomerizing rosin acids with HCl and purifying the abietic acid by recrystallization of the quarter salt of abietic acid. $[\alpha]_D^{20}$ —87° (12)

Dihydroabietic Acid — Prepared by hydrogenation of abietic acid and purified by recrystallization; m.p. 129-131° C.; $[\alpha]_D^{20}$ —3°.

Tetrahydroabietic Acid—Prepared by hydrogenating abietic acid and purifying by recrystallization. Negative to tetranitromethane test for unsaturation.

Fatty Acids and Natural Vegetable Oil Acids

Caprylic Acid—n-Caprylic acid from Eastman Kodak Co.

Lauric Acid—From Wecoline Products, Inc. Acid no. 272-275, Sap. no. 280, Iodine no. 1.5-2.0.

Myristic Acid — From Woburn Degreasing Co., technical myristic acid. Acid no. 237-243, Sap. no. 240-246, Iodine no. 4-13.

Palmitic Acid — Product from vacuum distillation of technical palmitic acid from J. T. Baker Chemical Co.

Stearic Acid — Wecoline Products, Inc. Acid no. 202-203, Sap. no. 203-204, Iodine value, less than 1.

Oleic Acid—Wecoline Products, Inc. Acid no. 199-201, Sap. no. 200-202, Iodine no. 88-89.

Linseed Oil Acids—Wecoline Products, Inc. Acid no. 198-200, Sap. no. 202, Iodine no. 179-182.

Coconut Oil Acids—Woburn Degreasing Co. Acid no. 260-270. Sap. no. 263-274, Iodine no. 9-15.

Palm Oil Acids—Woburn Degreasing Co. Acid no. 196-203, Sap no. 198-206, Iodine no. 40-55.

DUE to hydrolysis, rosin soaps, fatty acid soaps, and all usual household and toilet soaps give an alkaline reaction in water. Frequently household soaps contain builders. Bar soaps and soap flakes for household use in solutions at concentrations of 0.25 and 0.5 per cent were found to have pH values within the range of 10.0 to 10.4 when tested with a glass electrode (Beckman pH meter). At the concentrations and under the conditions that toilet soaps are usually employed the pH should be lower than the foregoing figures since hydrolysis of soap decreases as its concentration in solution increases (6).

Some workers (2, 8) have associated the germicidal action of soap with its hydrolysis and contend that the germicidal activity is due to the action of the hydroxyl ion formed. Others (8) have expressed doubt as to the influence of the hydroxyl ion formed by hydrolysis, inasmuch as its concentration is only on the order of 0.0001 N. From the chemical viewpoint it would appear that for strict comparisons as to the relative bactericidal activity of soaps derived from various organic acids, tests should be made within or close to the pH range governed by the dissociation of the alkali salts of the specific acid being studied, and not necessarily at a common pH level.

Experiments run at higher pH values may reflect common cation or hydroxyl ion activity. Tilley and Schaffer (15) have shown clearly that the activity of coconut oil soaps is increased by the action of a common cation. Cade and Halvorson (1) have found a similar action with the alkali salts of certain phenols and the bactericidal effect of the hydroxyl ion itself at relatively high concentrations (pH 12 or higher) is well established (10). Tests run at pH values lower than that characteristic of the alkali salt could be expected to reflect the activity of the aliphatic or rosin acid itself or the combined activity of a lowered concentration of the alkali salt and acid. A scrutiny of the methods employed in previous investigations on the germicidal activity of soap solutions reveals many examples of comparisons made at various arbitrarily selected pH levels outside the pH range characteristic of soaps. The value of such determinations is limited definitely to the specific pH at which they were made. In the absence of data showing the activity at the normal pH of the soap itself, it is difficult to say with certainty how useful the results are for comparative purposes. It is interesting to note that Walker (17, 18, 19), although reporting no pH measurements, pre-

pared his test soaps in a manner (which should have given him pH values) characteristic of the soaps studied. Thus, from a comparative standpoint, the works of Walker deserve special consideration.

Before conducting experiments on soap solutions, the germicidal activity of alkali and some alkaline salt solutions was determined to establish: (1) A knowledge of the resistance of *Staphylococcus aureus* to alkaline solutions; (2) the effect of temperature on the germicidal activity of alkaline solutions; (3) the effectiveness of 5 and 10 per cent alcohol solutions at an alkalinity comparable to that of soap solutions for killing a test organism. These studies were necessary preliminaries to the selection of the most suitable pH and temperature for testing and to provide evidence as to the suitability of methods of compounding test soap solutions.

All laboratory methods employed in the testing of germicides are necessarily more or less empirical. Frequently in vitro method results are expressed as coefficients determined by comparing the time or concentration necessary to completely kill selected organisms with that of an arbitrary standard, such as phenol. It was believed, however, that for direct comparative work with the materials at the concentrations to be studied, an accelerated death time method as employed by Cade and Halvorson (2) for testing soaps and alkaline detergents, or a slight modification of this method, would be more informative. Results obtained with this method are most easily expressed by survival time curves.

In all tests 15 ml. of the solution being studied was pipetted with a sterile pipette into sterilized lipped pyrex test tubes 25 by 150 mm. The tube containing the test material was immediately suspended in a thermostatically controlled constant temperature water bath and the contents allowed to come to the desired temperature. Transfer of the test organisms to the tube was made with a single standard loop. The specifications for this loop are given by Ruehle and Brewer (14). Using the same standard loop, the tube containing the solution being tested and the test organisms was sampled at intervals of 1, 2, 5, 10, and 20 minutes. These standard loop samples were transferred to tubes containing 15 ml. of sterilized nutrient agar maintained at 45° C. This agar was immediately poured into a sterile petri dish and allowed to solidify. As soon thereafter as possible the plates were placed in an incubator for 48 hours at 30° C. Counts were then made using a Quebec colony counting chamber.

Table 1.—Germicidal Action of Alkali and Alkaline Salt Solutions at 30° C. against Staphylococcus aureus.

Composition of solution	*ph of solution*	*Plate count* — *Exposure time in minutes* 1	2	5	10	20
H_2O (distilled)	6.8	4160	3840	3712	3712	3584
0.85% NaCl	7.0	4106	4160	4106	3840	4160
5% Alcohol (by volume) (+ Na_2CO_3)	10.1	3712	3840	3712	3776	3776
10% Alcohol (by volume) (+ Na_2CO_3)	10.1	3978	3904	4096	4352	4352
NaOH	10.0	2752	2432	2240	2048	1664
NaOH	11.0	3840	3072	3072	2304	2048
KOH	10.0	4480	3008	2752	2032	1792
KOH	11.0	3072	2560	2486	2496	1408
Na_2CO_3	10.1	2816	2304	2112	1920	1792
Na_2CO_3	11.1	2436	1856	1664	1664	1664
$Na_3PO_4.12H_2O$	10.0	2752	1920	1472	1152	768
$Na_3PO_4.12H_2O$	11.0	4096	3136	2752	2048	1664
$Na_2SiO_3.5H_2O$	10.0	2496	5000+	5000+	2048	1664
$Na_2SiO_3.5H_2O$	11.0	2560	2496	1984	1664	768

The composition of the nutrient agar employed was the same as that specified by Ruehle and Brewer (14) for the maintenance of stock cultures. The test organisms employed were *Staphylococcus aureus*, *Escherichia coli* and *Eberthella typhi*. Prior to use all test cultures were grown in plain nutrient broth for not less than 20 and not more than 24 hours at 37.5° C. The nutrient broth employed conformed to the specifications for plain broth established by the Food and Drug Administration of the U. S. Department of Agriculture for tests to determine phenol coefficients (14).

Preliminary tests with tap water, distilled water and physiological saline indicated that by following the procedure as outlined above the colony density on plates having the maximum number of colonies would not be great enough to introduce a large factor of error in counting when the Quebec colony counting chamber was employed. In most instances the tests have borne out this indication.

Control plates prepared by the method adopted using *Staphylococcus aureus* gave petri dish population density between 3,000 and 4,000 colonies. This is admittedly a very heavy seeding of colonies, but by employing the Quebec colony counting chamber quite precise counts could be obtained as judged by the ability of the operator to duplicate counts from the same plate.

The results of determinations with water, physiological saline, dilute alcohol solutions at pH 10, alkali and alkaline salt solutions having a pH of 10 and 11, and solutions of sodium hydroxide at pH values of 10, 11, 12, and 13 at temperatures ranging from 20° to 45° C., are given in Tables 1 and 2.

In Table 1 the counts indicate a slight though progressive death rate for the test organism in distilled water over a period of 20 minutes. In 0.85 per cent sodium chloride this slight death rate was not observed. The count after 20 minutes' exposure was as great as that found after a one-minute exposure. The counts in 5 and 10 per cent alcohol solutions made alkaline to pH 10.1 with sodium carbonate were equivalently as large, within the range of experimental error, as those found with 0.85 per cent sodium chloride and showed no progressive decrease with exposure time. It would appear, therefore, that

5 and 10 per cent alcohol in the soap solutions does not have any appreciable direct germicidal action.

The counts for solutions of sodium hydroxide, potassium hydroxide, sodium carbonate, trisodium phosphate and sodium metasilicate at pH 10.0 and 11.0 were, on the whole, slightly lower than those found for distilled water, 0.85 per cent sodium chloride, or the dilute alcohol—sodium carbonate solutions. With the exception of the sodium metasilicate solution at pH 10.0, all of these solutions showed small, though consistent, decreases in counts as the exposure time increased. The rate of this decrease appeared to be, in general, of the same order of magnitude at both pH 10 and 11. It would appear, then, that at 30° C. and these pH values, the alkalies and alkaline builders do exert a direct, though slight, bactericidal action.

The pronounced increase in count with the solution of sodium metasilicate at pH 10 after 2 and 5 minutes' exposure, followed by an abrupt decrease after 10 minutes' exposure is particularly interesting. This phenomenon was observed in repeated tests, and is apparently caused by a dispersing action on the natural grape-like groupings of staphylococci thus making the plate count higher. Microscopic stains made of smears from test solutions at the exposure intervals designated indicate a dispersing action. This suggests that dispersion may be one of the first phases in the killing action of certain alkalies. The high plate counts resulting from this dispersion indicate the empirical nature of the plate counts, since it shows plainly that the initial number of individual cells in the test suspension is actually far greater than the plate counts made from distilled water and physiological saline show.

A similar, though less pronounced and not quite so consistent, observation of this dispersing action with time was found at 30° C. with NaOH solutions at pH 11 (see Table 2). That the dispersing action is also influenced by concentration of alkali is clearly illustrated by the counts recorded by Table 2 at 30° C. and 35° C. for one minute at pH 10, 11, 12, and 13. Here the counts at pH 11 and 12 are much higher than those found at pH 10 and at pH 13 the counts show a marked drop. With the longer intervals of exposure the increased count is only observed at pH 11, whereas with the shorter time intervals it appears at both pH 11 and pH 12.

The experiments on the effect of pH and temperature on alkali germicidal activity show quite clearly that at 35° C. or lower the alkali does not show appreciable germicidal activity unless the pH is greater than 11. At 45° C. a marked germicidal action was observed at all of the pH values. From the results presented in Tables 1 and 2, the selection of a temperature of 30° C. and a pH value of about 10 for comparing the germicidal action of the soap solutions would be expected to minimize or almost eliminate the direct germicidal action of the elevated temperatures and hydroxyl ion.

Soap solutions for testing were prepared from the materials previously tested according to the following procedure:

Two grams of rosin, resin or rosin acid, fatty acid or mixture of fatty acids, was dissolved in 12 ml. of 95 per cent alcohol, 6 drops of phenolphthalein were added and the solution neutralized with approximately 2 N NaOH to the phenolphthalein end point. This solution was poured into 50 ml. of approximately 0.01 per cent sodium carbonate and then diluted to 200 ml. with 0.01 per cent sodium carbonate. This procedure gave soap solutions with pH values between 10.0 and 10.4. For soap solutions with a pH of 11, 0.2 per cent sodium carbonate was used in place of the 0.01 per cent sodium carbonate. Soap solutions containing 0.5, 0.25 and 0.1 per cent rosin or fatty acids were prepared by diluting the solution containing 1 per cent rosin or fatty acid with the proper amount of sodium carbonate solution.

To determine the influence of 0.01 per cent sodium carbonate on the germicidal activity of soap solutions, tests were made on 0.25 per cent solutions with and without the added buffer. The results for sodium laurate are given in Table 3.

No appreciable difference was found between the germicidal activity of soap solutions with and without 0.01 per cent sodium carbonate. The pH of 0.1 to 1.0 per cent aqueous solutions of the sodium salts of the

Table 2.—Effect of Temperature on the Germicidal Action of Sodium Hydroxide Solutions Against Staphylococcus aureus.

pH of solution	*Temperature of solution*	*Plate count*[1]				
		Exposure time in minutes				
		1	2	5	10	20
	degrees C.					
10	20	2304	1728	1728	1792	1728
10	30	1856	1664	1600	1472	1344
10	35	1600	1600	1536	1536	1472
10	40	1536	1472	1152	1152	960
10	45	1472	768	312	188	4
11	20	1728	1728	1728	2688	2368
11	30	3200	3264	3456	3968	3435
11	35	2048	1536	1536	1536	1536
11	40	1536	1408	1408	1088	300
11	45	1152	608	138	6	0
12	20	1792	1984	1728	384	118
12	30	3008	2048	2048	78	8
12	35	3072	2176		6	0
12	40	1408	1216	1216	6	0
12	45	11	0	0	0	0
13	20	90	5	1	1	1
13	30	100	9	2	0	0
13	35	22	0	0	0	0
13	40	3	1	0	0	0
13	45	0	0	0	0	0

[1] Control plate count 3 to 4 thousand.

Table 10.—Germicidal Activity at 30° C. of Fatty Acid and Fatty Acid-Rosin Soap Solutions at pH 10.2 against *Escherichia coli.*

Soap Solution	Ratio of Components by Weight	Plate Count[1] — Concentration of Solutions: 1.0 Per Cent — Exposure time in minutes 1	2	5	10	20	0.5 Per Cent — Exposure time in minutes 1	2	5	10	20	0.25 Per Cent — Exposure time in minutes 1	2	5	10	20
Made from																
Slash pine Rosin[2]	....	576	5	2	0	0	616	512	124	56	..	1792	1088	960	640	..
Coconut oil Acids.....	....	18	5	0	0	0	704	515	256	256	256	768	512	204	128	96
Palm Oil Acids.....	....	2240	2240	2240	2240	2240	2240	1920	1920	1920	1920	2432	2432	2432	2432	2432
Palm Oil Acids— Coconut Oil Acids...	3-1	1984	1600	512	384	176	2304	1920	1600	1408	896	2660	2660	2660	2660	2304
Palm Oil Acids— Slash pine Rosin....	3-1	2368	1984	1216	264	58	2304	2304	1984	1920	832	1546	1280	1088	704	640
Coconut Oil Acids— Slash pine Rosin....	3-1	7	0	0	0	0	308	138	24	12	8	448	370	264	42	24
Palm Oil Acids— Coconut Oil Acids— Slash pine Rosin....	2-1-1	1600	768	82	8	7	704	448	104	48	41	1024	632	768	586	320

[1] Control plate count approximately 3,000.
[2] Slash pine rosins used in all combinations.

indicated less germicidal activity than when the gels were melted at 55° C. and cooled to 30° C. and then tested. Activity appeared to decrease with time with test solutions that tend to set to gels at 30° C. In these studies all such soap solutions were heated to 55° C. and then cooled to 30° C. and tested as soon thereafter as they came to temperature. In this way the effect of the physical state of the solution has been minimized.

The influence of temperature on the germicidal activity of soap solutions is of interest when considering possible results in connection with a particular use. The data from a few tests on the effect of temperature on the germicidal activity of selected soaps against *Staphylococcus aureus* are given in Table 11.

The counts in Table 11 emphasize the desirability of using 35° C. or less and accurately controlling the temperature for comparative bactericidal tests on soap solutions. When temperatures higher than 30° C. are used the germicidal activities of the soaps appear to approach each other, and the differences between the various soaps are more difficult or impossible to determine with certainty. At temperatures of 40° C. or higher, all of the soap solutions become more germicidal in the concentrations tested.

Some of the data presented in tables 2, 4, 5, 6, 7, 9, 10, and 11

Table 11.—Effect of Temperature on Germicidal Activity of Soap Solutions at pH 10.2 against *Staphylococcus aureus.*

Soap solutions	Concentration of solution	Ratio of components by weight	Plate Count[1] after exposing to soap solution for 5 min. at[2] 25°	30°	35°	40°	45°
Made from	*Per Cent*						
Palm oil acids— coconut oil acids.....	1.0	3-1	184	76	2	1	0
Palm oil acids— coconut oil acids.....	0.5	3-1	188	92	16	2	0
Palm oil acids— coconut oil acids.....	0.25	3-1	284	94	34	3	2
Palm oil acids.....	1.0	...	288	146	33	4	0
Palm oil acids— coconut oil acids.....	1.0	3-1	184	76	2	1	0
Palm oil acids— coconut oil acids— rosin	1.0	2-1-1	117	26	2	1	0
Sodium hydroxide — pH 10.0			...	1664	1536	1152	312

[1] Control plate count 3 to 4 thousand.
[2] Degrees C.

have been graphically analyzed. In Plates 1, 2, and 3 graphs are presented. In these figures the log of the number of surviving bacteria is plotted against the factors of time, temperature, pH and concentration. The rate of the death of bacterial cells as influenced by germicides is usually considered to be a logarithmic function of the initial number of organisms present.

In Plate 1 survival curves of *Staphylococcus aureus* with time for 0.5 per cent solutions of palm oil, linseed oil, coconut oil and slash rosin soaps are plotted. The superior germicidal activity of the slash rosin soap over these natural oil soaps is obvious. In plate 1, figure 2, the survival curves of *Staphylococcus aureus* with time for 0.25 per cent solutions of the purified fatty acid soaps may be compared with that of a purified abietic acid soap. Here the superiority of the rosin acid soap over fatty acid soaps is plainly indicated. In plate 1, figure 3, the survival curves of *Staphylococcus aureus* with time for 0.25 per cent solutions of the soaps made from normal gum rosin, oxidized gum rosin and pyroabietic acid plainly indicates the differences in germicidal activity of these products as previously mentioned in the text. In plate 1, figure 4, the differences are shown in the relative germicidal activities of rosin acid soaps of different structure at a concentration of 0.1 per cent as tested against *Staphylococcus aureus.*

In plate 2, figures 1 and 2 show the survival curves for *Staphylococcus aureus* as influenced by in-

creasing the pH of sodium hydroxide solutions and 0.25 per cent solutions of linseed oil and slash rosin soaps from 10 to 11. Here the increase in the germicidal activity of soap solutions with the higher pH values is strikingly illustrated. In plate 2, figures 3 and 4 show the effect of temperature and pH on the germicidal activity of aqueous solutions of sodium hydroxide. These effects may be more clearly visualized from graphs than from the prior tabular presentation and the text discussion.

Plate 3, figure 1 shows the relative germicidal activity of the soaps of slash rosin, oxidized rosin and pyroabietic acid at concentrations ranging from 0.1 ot 2.0 per cent. The data for tests made at 0.1 and 2.0 per cent were not included in the tables in the text. This figure shows that the relative effectiveness of these three soaps against *Staphylococcus aureus* as brought out for one concentration in plate 1, figure 3 holds good over the range of concentrations tested at a given time interval of exposure.

In plate 3, figures 2 and 3 some of the survival curves with time for the soap mixtures studied against both *Escherichia coli* in concentrations of 1 per cent and *Staphylococcus aureus*, in concentrations of 0.5 per cent, respectively, are given. Here the increase in the germicidal activity of the fatty acid soaps by the addition of rosin soap is plainly shown by the relative position of the curves.

Plate 3 figure 4 shows the greater killing action of rosin soap compared to coconut oil soap at all concentrations when *Staphylococcus aureus* was the test organism. The superimposition of the death curves for *Escherichia coli* with slash rosin and coconut oil soap with increasing concentrations at a 2-minute exposure time brings out the equivalent activity of these two soaps against this organism.

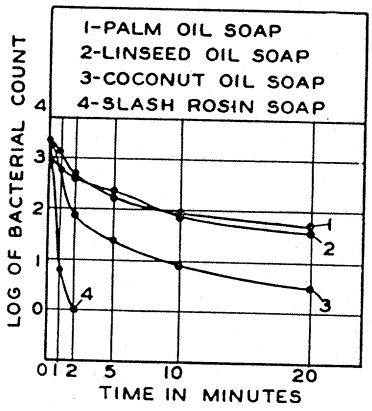

FIGURE 1

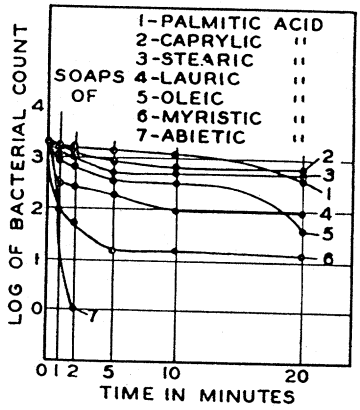

FIGURE 2

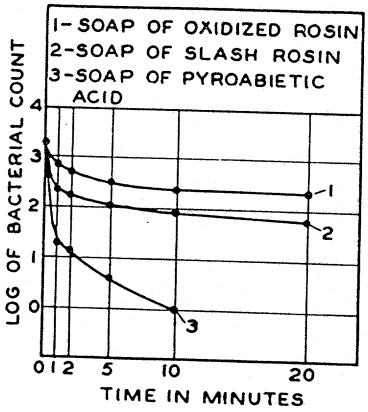

FIGURE 3

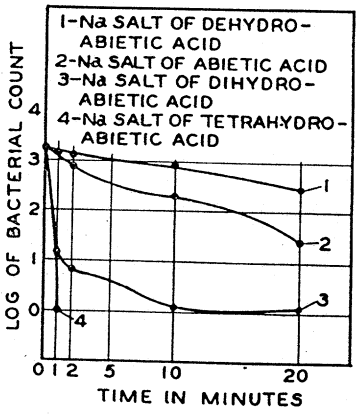

FIGURE 4

PLATE 1

Fig. 1—Relative germicidal activities of some natural oil soaps and rosin soap in concentrations of 0.5 per cent against **Staphylococcus aureus,** at 30° C.

Fig. 2—Relative germicidal activities of some pure fatty acid soaps and sodium abietate in concentrations of 0.25 per cent against **Staphylococcus aureus,** at 30° C.

Fig. 3—Relative germicidal activities of soaps made from slash rosin, oxidized rosin, and pyroabietic acid in concentrations of 0.25 per cent against **Staphylococcus aureus,** at 30° C.

Fig. 4—Relative germicidal activities of the sodium salts of certain resinic acids in concentrations of 0.1 per cent against **Staphylococcus aureus,** at 30° C.

Discussion

The value of a zero count in the method employed has been discussed by Halvorson and Zeigler (7). In these studies zero counts were rarely ever followed by any counts other than zero in any series of exposure intervals. In fact, the infrequent appearance of so-called "skips" was a noteworthy feature of this investigation. Thus, where a zero count is recorded, followed by a continuous succession of zero counts at the longer intervals, it appears safe to interpret the results as complete killing.

Solutions of soaps made with rosin alone in a concentration of 2 per cent will kill the three organisms studied. The combination of soap properties, germicidal action, and the fact that rosin soaps do not become rancid with time may make rosin soap a useful germicide and cleanser for many purposes.

That the inclusion of rosin or rosin acid soaps in commercial soaps would tend to increase their germicidal activity, has been plainly indicated.

Predicting the results a soap containing rosin will give, when used

for washing the hands, dishes, or in the home laundry, involves factors which cannot be accurately evaluated from test tube experiments.

A more complete investigation of the specificities of the soaps of rosin and individual rosin acids for different organisms might reveal special uses for some of these products.

Conclusions

The following conclusions were drawn from the results of this study against *Staphylococcus aureus*, *Escherichia coli*, and *Eberthella typhi*.

Rosin soap solutions were more active as germicides than equivalent concentrations of fatty acid soap solutions.

Mixtures of rosin soaps and fatty acid soaps were germicidally more active than the corresponding fatty acid soaps.

Soaps made from rosins produced from longleaf pine gum and slash pine gum, respectively, had equivalent germicidal activity.

Oxidation of the unstable rosin acids reduced the germicidal activity of the resulting rosin soap.

Soaps made from pyroabietic acid prepared as described were more active as germicides than soaps made from gum rosins.

The sodium soaps of tetrahydro-, dihydro-, and freshly prepared abietic acids were more active as germicides than soaps made from gum rosins.

Coconut oil soap was germicidally more active against *Escherichia coli* and *Eberthella typhi* than other soaps made from natural fats and oils. Against these two test organisms coconut oil soap had an activity equivalent to that of a normal gum rosin soap. Rosin soap was much more active germicidally than coconut oil soap when *Staphylococcus aureus* was used as the test organism.

Soaps made from lauric or myristic acids, the two principal constituents of coconut oil were more active against *Escherichia coli* and *Eberthella typhi* than coconut oil soap.

The germicidal activity of the soaps of the purified rosin acids studied increased with the amount of hydrogen in the rosin acid molecule when *Staphylococcus aureus* was used as the test organism. However, this correlation of germicidal activity with the molecular structure did not hold when *Escherichia coli* was used as the test organism.

Increasing the pH of both rosin soap solutions and fatty acid soap solutions above that normal to the household and toilet soaps increased the germicidal activity of these solutions.

Solutions of alkalies and alkaline salts in concentrations that have a pH of 10 and 11 were only slightly germicidally active. Sodium hydroxide solutions did not become markedly germicidally active until the pH of the solutions exceeded 11 or the temperature was raised above 40° C.

The germicidal activity of all soap solutions increased as the tem-

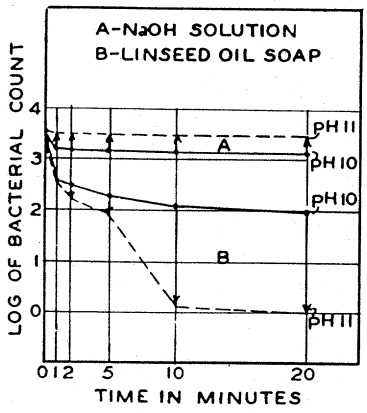

FIGURE 1

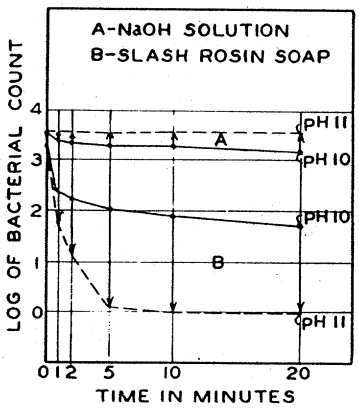

FIGURE 2

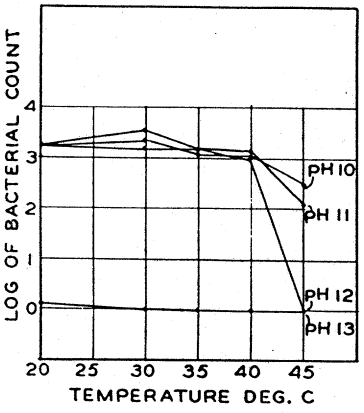

FIGURE 3

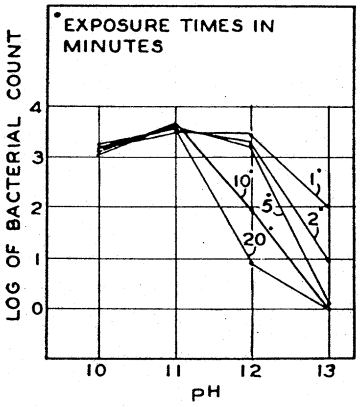

FIGURE 4

PLATE 2

Fig. 1—Effect of increasing the pH from 10 to 11 on germicidal activity of 0.25 per cent linseed oil soap at 30° C. against **Staphylococcus aureus.**

Fig. 2—Effect of increasing the pH from 10 to 11 on germicidal activity of 0.25 per cent rosin soap at 30° C. against **Staphylococcus aureus.**

Fig. 3—Effect of temperature on germicidal activity of solutions of NaOH at pH 10-11-12 and 13 as shown by 5-minute exposures of **Staphylococcus aureus.**

Fig. 4—Effect of increasing pH on germicidal activity of NaOH solutions at 30° C. against **Staphylococcus aureus.**

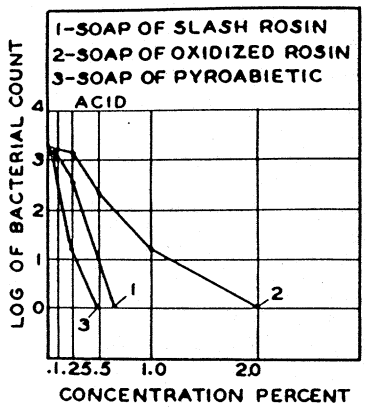

FIGURE 1

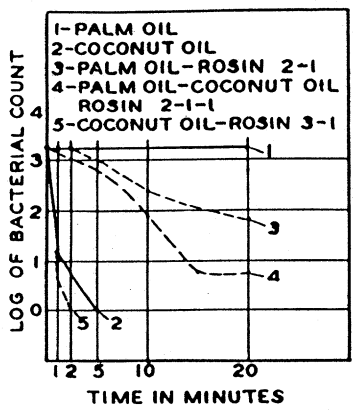

FIGURE 2

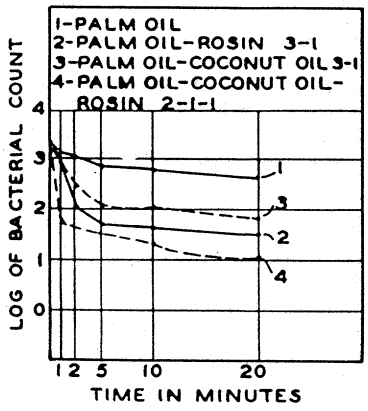

FIGURE 3

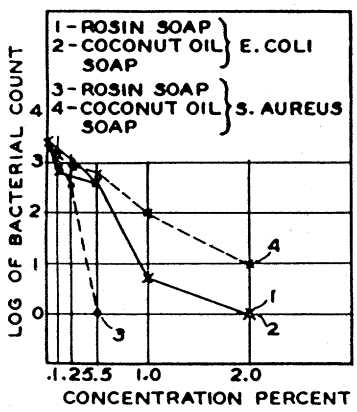

FIGURE 4

PLATE 3

Fig. 1—Relative germicidal activity of rosin soap for 2 minutes exposure of **Staphylococcus aureus** at 30° C. and various concentrations.

Fig. 2—Relative germicidal activity of 1.0 per cent fatty acid and fatty acid-rosin soap solutions against **Escherichia coli** at 30° C.

Fig. 3—Relative germicidal activity of 0.5 per cent fatty acid and fatty acid-rosin soap solutions against **Staphylococcus aureus** at 30° C.

Fig. 4—Comparison of germicidal activity of rosin soap and coconut oil soap solutions against **Staphylococcus aureus** and **Escherichia coli,** exposure time 2 minutes.

of *Escherichia coli* was accomplished in 2 minutes at 30° C. with a 2 per cent solution of either rosin soap or coconut oil soap at pH 10.2.

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Germicidal Activity Of Soaps

A study of the comparative germicidal value of rosin soaps and soaps of individual fatty acids

Part II

By L. S. Stuart and W. D. Pohle

SIMILAR studies were made on most of the soaps tested against *Staphylococcus aureus* using *Escherichia coli* as the test organism. Counts made from standard loop suspensions of 24-hour cultures of this organism, in 15 ml. of physiological saline, indicated that the initial inoculum of the test solution was between 2500 and 3500 per standard loop subsample. This was less than was found with *Staphylococcus aureus*. Typical plate counts for tests with this organism are given in Table 7.

The results with *Escherichia coli* did not show quite such a consistent picture of greater germicidal activity for the rosin soaps when compared with fatty acid soaps at concentrations of 1.0 and 0.5 per cent. With this test organism equivalent concentrations of coconut oil soap appear to have a germicidal activity equal or nearly equal to rosin soap made from normal gum rosin. The soaps of lauric and myristic acids are more effective than the other fatty acid soaps tested. The tests with pyroabietic acid indicate an effectiveness about equivalent to that of lauric and myristic acid.

The results obtained with purified rosin acids were somewhat erratic when this test organism was employed. The order of activity of the rosin acids appears to be the reverse of that found with *Staphylococcus aureus*, but the differences observed were not great or consistent at the concentration employed. Accurate duplication of counts with this organism was more difficult than with *Staphylococcus aureus*, and therefore small differences are probably of little significance.

The apparent reversal of the order of activity of the pure rosin acid soaps with *Escherichia coli* as compared to the results with *Staphylococcus aureus* as the test organism indicates that the soaps of the various rosin acids may themselves have some specificities for different organisms similar to those observed and reported for the various fatty acid soaps by Walker (17, 18, 19) and Eggerth (3).

To establish definitely the similarity of the resistance of the strains of *Escherichia coli* used in this study with that of *Eberthella typhi*, some direct comparisons were made between the resistance of 24-hour cultures of the two organisms to selected rosin and fatty acid soaps and phenol. The results of these tests are presented in Table 8.

From the counts in Table 8 it would appear that the resistance of the culture of *Escherichia coli* used to the germicidal action of the

Table 7.—Germicidal Activity at 30° C. of Rosin and Fatty Acid Soap Solutions at pH 10.2 against *Escherichia coli*.

Plate Count[1]

Soap Solution	Concentration of Solutions: 1.0 Per Cent — Exposure time in minutes 1	2	5	10	20	0.5 Per Cent — Exposure time in minutes 1	2	5	10	20	0.25 Per Cent — Exposure time in minutes 1	2	5	10	20
Made from															
Slash pine Rosin	576	5	2	0	0	616	512	124	56	..	1792	1088	960	640	..
Pyroabietic Acid	[2]	0	0	0	0	..	12	11	6	..	2048	512	288	96	..
Dehydroabietic Acid	3	0	0	0	0	..	384	384	304	..	1664	704	448	320	..
Abietic Acid	144	0	0	0	0	304	148	53	24	..	1328	704	448	320	..
Dihydroabietic Acid	88	7	0	0	0	..	400	104	48	..	..	960	960	852	..
Tetrahydroabietic Acid	640	512	10	0	0	1792	1216	960	640	448	1536	1280	1216	1152	1152
Caprylic Acid	960	640	57	3	0	1536	1472	832	576	384	2438	2438	2438	2240	2240
Lauric Acid	6	0	0	0	0	64	53	89	16	9	832	640	512	512	512
Myristic Acid	32	20	4	0	0	374	108	74	26	17	460	640	448	148	98
Oleic Acid	..	52	54	23	..	1280	832	576	448	384	1984	1728	1664	704	704
Stearic Acid	..	..	..	..	..	..	..	..	..	..	2816	2816	2816	2816	2304
Palm Oil Acids	2240	2240	2240	2240	2240	2240	1920	1920	1920	1920	2437	2432	2432	2432	2432
Coconut Oil Acids	18	5	0	0	0	704	512	256	256	256	768	512	204	128	96
PHENOL[3]	0	0	0	0	0	2304	2112	896	96	32	3585	3392	3392	3200	3520

[1] Control plate count approximately 3,000.
[2] Not run at the concentration or exposure interval indicated by..
[3] Phenol tested in water solution at pH 6.2 made up and standardized according to Ruehle and Brewer (14).

Table 8.—Germicidal Activity at 30° C. of Rosin and Fatty Acid Soap Solutions at pH 10.2 against *Escherichia coli* and *Eberthella typhi*.

Soap solution	Concentration of solution	Plate count: *Escherichia coli* — Exposure time in minutes 2	5	10	Plate count: *Eberthella typhi* — Exposure time in minutes 2	5	10
Made from	*Per cent*						
Slash pine Rosin	2	0	0	0	0	0	0
Coconut Oil	2	0	0	0	0	0	0
Slash Rosin	1	5	2	0	2	0	0
Coconut Oil	1	5	0	0	3	2	0
Oleic Acid	1	576	192	6	52	54	23
Pyroabietic Acid	1	0	0	0	0	0	0
Palm Oil	1	2240	2240	2240	888	472	388
NaCl[1]	0.85	2432	2112	2112	2560	2560	2560
Phenol[2]	1	0	0	0	0	0	0
Phenol[2]	0.5	2112	896	96	1680	384	320

[1] Physiological saline, pH 7.0.
[2] Phenol tested in water solution at pH 6.2. Standardized according to Ruehle and Brewer (14).

materials studied was essentially the same as that of *Eberthella typhi*. Both organisms were completely killed by 2 per cent coconut oil soap and 2 per cent rosin soap. The resistance of both organisms to 1.0 per cent solutions of the soaps of rosin, coconut oil, oleic acid, pyroabietic acid, and palm oil was about the same. Both organisms were completely killed by 1.0 per cent phenol, yet both were not killed by 0.5 per cent phenol at pH 6.2.

The results in Tables 7 and 8 on the whole indicate that soap made with normal gum rosin has about the same order of activity against *Escherichia coli* and *Eberthella typhi* as coconut oil soap. The sodium soaps of pyroabietic acid, lauric acid, and myristic acid are slightly more effective than either the soap of gum rosin or coconut oil.

In the manufacture of soaps containing rosin soap the ratio of fatty acid to rosin may be as much as 2 to 1. Since rosin is always used with fatty acids in household soaps, the germicidal activity of fatty acid-rosin soaps, compared to fatty acid and rosin soaps individually, is of interest. Tables 9 and 10 give the results from tests made upon several mixtures of fatty acids and rosin in the form of soap against *Staphylococcus aureus* and *Escherichia coli*. These tests indicate that a soap solution containing fatty acids and rosin in the ratio of 3 to 1 is more active against *Staphylococcus aureus* than one containing fatty acids alone. At concentrations of 0.5 and 0.25 per cent the influence of the rosin (slash pine rosin) on the germicidal activity of the soap solution is less, diminishing as the concentration of the soap acids is reduced.

With *Escherichia coli*, the specificity of coconut oil soap observed in the previous tests again appears. The addition of coconut oil and slash pine rosin soaps to palm oil soap increases its germicidal activity to about the same extent. However, the addition of slash pine rosin soap to coconut oil soap definitely increased the germicidal activity of the coconut oil soap. There is no apparent explanation for this increase in the activity of coconut oil soap with the addition of rosin soap when *Escherichia coli* is used as the test organism. The observation has been verified and may be due to the effect of rosin soap on certain of the physical properties of the solutions.

Some difficulty was encountered in these studies in obtaining checks with the same soap solutions which contained palm oil, palmitic, or stearic acids in concentrations that tended to gel on standing at room temperature. The rate of setting of the gel, state of gel, and age of the gel all seemed to exert some influence on germicidal activity. Tests made on semi-rigid gels brought up to 30° C. in a water bath usually

Table 9.—Germicidal Activity at 30° C. of Fatty Acid and Fatty Acid-Rosin Soap Solutions at pH 10.2 against *Staphylococcus aureus*.

Soap Solution	Ratio of Components by Weight	Plate Count[1] — Concentration of Solutions 1.0 Per Cent — Exposure time in minutes 1	2	5	10	20	0.5 Per Cent — Exposure time in minutes 1	2	5	10	20	0.25 Per Cent — Exposure time in minutes 1	2	5	10	20
Made from																
Slash pine Rosin[2]		1	0	0	0	0	5	0	0	0	0	238	118	51	32	15
Coconut Oil Acids		23	8	8	6	2	104	76	39	23	10	248	116	88	57	12
Palm Oil Acids		2416	312	288	252	252	1152	1152	704	640	512	1408	1408	1152	768	704
Palm Oil Acids Coconut Oil Acids		576	320	111	97	36	640	256	127	124	54	704	502	438	134	56
Palm Oil Acids— Slash pine Rosin	3-1	176	9	6	1	1	704	92	48	42	..	320	244	132	96	26
Coconut Oil Acids— Slash pine Rosin	3-1	20	15	0	0	0	11	3	3	0	0	..	92	20	20	10
Palm Oil Acids— Coconut Oil Acids— Slash pine Rosin	2-1-1	47	24	12	11	7	52	48	23	12	13	211	136	110	66	24

[1] Control plate count 3 to 4 thousand.
[2] Slash pine rosins used in all combinations.

ing upon the individual acid and concentration. Sodium laurate and resinate have characteristic pH values lower than those of the principal fatty acid soaps found in the usual commercial soaps. Thus, the influence of the common ion added with the 0.01 per cent sodium carbonate buffer was as great with those materials as with any of the materials studied. Increasing the pH of these soaps to 10.2 with Na_2CO_3 did not alter appreciably their germicidal properties. The addition of 0.01 per cent sodium carbonate to sodium stearate and sodium palmitate, which have pH values without the addition of buffer slightly higher than 10, did not affect appreciably the pH values. Therefore, for the sake of uniformity sodium carbonate was used in all solutions. The small common ion effect was the same for all solutions.

To determine the influence of sodium carbonate buffer at the higher concentration of 0.2 per cent, and thus at the corresponding higher pH [illegible] sodium oleate were tested at about pH 10 and 11 using 0.01 and 0.2 per cent sodium carbonate, respectively. The results of this study, as shown in Table 4, indicate that increasing the pH value from approximately 10 to 11 does increase the germicidal action of the three soaps at the concentrations tested.

Care was taken, therefore, not to exceed pH values common to 1.0, 0.5, and 0.25 per cent solutions of commercial soaps. The germicidal activity under these conditions should reflect primarily the activity of the soaps themselves.

Fatty acids and rosin acid soap solutions were prepared, as described, with sodium carbonate at pH 10.2 and tested at 30° C. using *Staphylococcus aureus* as the test organism. Typical data from determinations on solutions containing 1.0, 0.5, and 0.25 per cent fatty acid, rosin or rosin acids in the form of soap are given in Table 5.

Table 3.—Influence of 0.01 per cent Sodium Carbonate Buffer on Germicidal Action of Sodium Laurate and Sodium Resinate, using Staphylococcus aureus as the test organism.

Soap solution	*Concentration of solution*	*pH of solution*[2]	*Plate count*[1] *Exposure time in minutes* 2	5	10	20
	Per cent					
Sodium laurate with buffer	0.25	10.2	256	192	92	88
Sodium laurate without buffer	0.25	9.2	248	144	108	47
Sodium resinate with buffer	0.25	10.2	184	105	85	52
Sodium resinate without buffer	0.25	9.4	368	206	138	63

[1] Control plate count 3 to 4 thousand.
[2] pH measured with a glass electrode (Beckman pH meter).

Table 4.—Effect of pH on the Germicidal Activity at 30° C. of Soap Solutions buffered with Sodium Carbonate using Staphylococcus aureus.

Soap solution	*Concentration of solution*	*pH of solution*	*Plate count*[1] *Exposure time in minutes* 1	2	5	10	20
Made from	*Per cent*						
Slash pine Rosin	0.25	10.0	251	184	105	85	52
Slash pine Rosin	0.25	11.1	42	10	1	0	0
Lauric Acid	1.0	10.0	22	12	9	8	6
Lauric Acid	1.0	11.1	52	17	5	0	0
Oleic Acid	1.0	10.2	576	20	3	2	2
Oleic Acid	1.0	11.1	196	29	1	0	0

[1] Control plate count 3 to 4 thousand.

that with *Staphylococcus aureus* as the test organism the rosin soap solutions at concentrations of 1.0 and 0.5 per cent have greater germicidal activity than equivalent concentrations of fatty acid soaps.

With this organism there is little or no difference in the bactericidal activity of rosin soap solutions made from either longleaf or slash pine rosin at concentrations of 1.0, 0.5, and 0.25 per cent. Soap solutions made from oxidized rosin are less germicidal than soaps made from normal gum rosin. Rosin soap made with pyroabietic acids is more active than that made with normal gum rosin. In 0.25 per cent solution the sodium salts of abietic†, dihydroabietic and tetrahydroabietic acids are more effective in killing the test organism than the other rosin soap solutions. The sodium salt of dehydroabietic acid was definitely less effective as a germicide at 1.0, 0.5, and 0.25 per cent concentrations than those of abietic, dihydroabietic or tetrahydroabietic acids. The salts of the last named acids were so active at concentrations of 1.0, 0.5 and 0.25 per cent that no distinction could be made. Considerable variation is shown in the activity of the fatty acid soaps made from individual fatty acids. Similar variations have been pointed out by previous workers (8, 18).

With *Staphylococus aureus* as the test organism, the soaps of oleic and linseed oil acids were slightly more effective than coconut oil soap. This agrees with the observations by Walker (18) and Cade and Halvorson (2) that the soaps of the unsaturated fatty acids were active against this organism. The soaps of caprylic, stearic and palmitic acids were less effective. The soaps of myristic and lauric acids were relatively more active against this organism than previous reports seem to have indicated.

For purposes of comparison with a germicide of known activity, results with equivalent concentrations of phenol (pH 6.2) were included in

† Rosin soap solutions made with abietic acid were tested when prepared as the germicidal activity decreased on standing, due no doubt to oxidation.

solutions are more active as germicides under the conditions of these tests than equivalent concentrations of aqueous phenol.

To determine the comparative activity of the rosin soaps made from dehydroabietic, dihydroabietic and tetrahydroabietic acid against *Staphylococcus aureus*, tests were run on 0.1 per cent solutions. These results are presented in Table 6.

The results in Table 6 show that the order of increasing germicidal activity of these rosin acid soaps is as follows: Dehydroabietic, abietic, dihydroabietic, tetrahydroabietic. With this organism, there seems to be a direct correlation between the amount of hydrogen in the rosin acid molecule and germicidal activity.

Table 6.—Comparative Germicidal Action at 30° C. of Rosin Soap Solutions at pH 10.2 against Staphylococcus aureus.

Soap solution	*Concentration of solution*	*Plate count*[1] *Exposure time in minutes* 1	2	5	10	20
Made from	*Per cent*					
Dehydroabietic Acid	0.1	1626	1280	868	764	320
Abietic Acid	0.1	1152	704	280	180	29
Dihydroabietic Acid	0.1	15	7	4	1	2
Tetrahydroabietic Acid	0.1	0	0	0	0	0

[1] Control plate count 3 to 4 thousand.

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A bleaching and cleansing agent which reduces and dissolves iron compounds in an alkaline or neutral solution has been announced by General Dyestuff Corp., New York. The compound, called "Burmol Extra," is a soluble white powder. In aqueous solution it is slightly alkaline and is stable for some time. Applications are for removing rust spots from white goods or for treating spotted or yellowed white goods during or after the washing process, and for bleaching textiles which are discolored by contact with water containing iron. The compound is used in 0.2-2 per cent solution. With alkali-sensitive materials such as wool and silk the product is used at a low temperature but with cotton goods it may be added to hot solutions. Rinsing should follow the treatment. *Textile World* 90, 92 (1940).

Table 5.—Germicidal Activity at 30° C. of Rosin and Fatty Acid Soap Solutions at pH 10.2 against *Staphylococcus aureus*.

Soap Solution	*Plate Count*[1] *Concentration of Solutions* 1.0 *Per Cent* *Exposure time in minutes* 1	2	5	10	20	0.5 *Per Cent* *Exposure time in minutes* 1	2	5	10	20	0.25 *Per Cent* *Exposure time in minutes* 1	2	5	10	20
Made from															
Slash pine Rosin.....	12	0	0	0	0	6	0	0	0	0	251	184	105	85	52
Longleaf pine Rosin.....	0	0	0	0	0	5	0	0	0		238	118	51	32	15
Oxidized Rosin	0	0	0	0	0	46	31	41	2	1	616	528	368	252	240
Pyroabietic Acid	1	0	0	0	0	0	0	0	0	0	20	19	3	0	0
Dehydroabietic Acid	28	0	0	0	0	49	22	5	4	1	584	520	320	248	136
Abietic Acid	2	1	0	0	0	0	0	0	0	0	10	0	0	0	0
Dihydroabietic Acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tetrahydroabietic Acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caprylic Acid 1344	832	768	512	384	704	640	640	640	640	1472	1349	906	768	704
Lauric Acid	22	22	9	8	6	44	6	5	2	3	256	256	192	92	88
Myristic Acid	42	38	38	37	37	78	52	48	42	9	93	58	19	16	11
Palmitic Acid	[2]	..	..	..	..	2112	1472	832	412	264	2048	2048	1344	1280	400
Stearic Acid	[2]	..	..	..	..	1920	1826	1762	1536	1536	1280	1024	502	502	502
Oleic Acid 576	20	3	2	2	768	512	77	4	4	960	768	384	320	36
Linseed Oil Acids..... 448	124	8	8	0	512	448	246	76	55	2048	1920	704	448	77
Coconut Oil Acids..... 258	156	78	76	34	704	576	224	142	92	832	832	576	384	124
Palm Oil Acids.....1024	448	84	28	23	1024	512	182	92	50	640	440	256	94	37
PHENOL[3] 114	7	0	0	0	1664	1408	1036	176	20	1664	1664	1664	1600	640

[1] Control plate count 3 to 4 thousand.
[2] Not tested due to formation of rigid gel at this concentration.
[3] Phenol tested in water solutions pH 6.2 standardized according to Ruehle and Brewer (14).